

Project Title: Comparing Pressure-Retained and Decompressed Microbial Communities from the Greatest Ocean Depths**PROJECT REPORT**

Hadal trenches are one of the least studied portions of the biosphere. These sites, found deeper than 6500 m, are thought to be home to microbial communities unique from those at shallower depths. Hadal microbes are expected to be adapted to high hydrostatic pressures, low temperatures, and low and recalcitrant forms of organic matter. Therefore cell viability and microbial activity may be higher under *in situ* pressures, but such measurements are lacking. Deep-ocean samples are typically collected with Niskin bottles under decompressed conditions. Therefore we developed a pressure-retaining sampler (PRS) capable of collecting hadal water and maintaining *in situ* pressure upon recovery.

To estimate the microbial abundance, diversity, and activity as a function of decompression, water samples were collected from the Atacama Trench aboard the *RV Sonne* during cruise SO261. This included eleven attempted deployments of the pressure-retaining sampler, which held between 60-70% of the *in situ* pressure. Sampled depths included 50 m, 500 m, 2500 m, 5000 m, and 7000-8000 m to evaluate different oceanographic regimes (surficial, mesopelagic, bathypelagic, abyssopelagic, and hadopelagic) for their pressure response.

Laboratory analyses of the samples collected is ongoing. Activity of microbial communities within samples obtained using the pressure-retaining sampler will be compared against those collected using Niskin bottles which were 1) recompressed to *in situ* pressure 2) recompressed to 80 MPa, full trench depth 3) maintained under decompressed conditions. Samples were both fixed with formaldehyde for total counts and preserved with glycerol-TE for single-cell sorting. Decompressed samples were also concomitantly incubated at room temperature and the aforementioned conditions for two and five days to determine how the pressure sensitivity of these samples changes as a function of temperature and length of incubation. Estimates of microbial activity will be evaluated using fluorescence microscopy and FACS-based single-cell genomics to identify active populations under different pressure conditions. Pressure-retained samples will be also be used for culturing at SIO. Preliminary results indicate that cell numbers are higher when samples are fixed under *in situ* conditions, suggesting that some deep-ocean microbes may lyse during decompression. This has fundamental implications for how we sample the deep ocean.

Table 1, below. Sample collection locations within the Atacama Trench.

Date	Station	Deployment name	Latitude (S)	Longitude (W)	Maximum Water	Sampled Depths	PRS deployed?
					Depth (m)		
3/5/18	Station 1	SO261-7	23° 49.248' S	070° 50.164' W	2549.1	2400, 50	-
3/7/18	Station 6	SO261-19	24° 17.042' S	071° 25.401' W	7830.4	7500	Yes
3/7/18	Station 6	SO261-13	24° 16.082' S	071° 25.414' W	7837.8	5000, 2500, 500, 50	-
3/9/18	Station 6	SO261-24	24° 16.773' S	071° 25.390' W	7834.1	7000	Yes
3/10/18	Station 5	SO261-28	23° 49.121' S	071° 22.351' W	7873.6	500, 2500, 5000	-
3/11/18	Station 5	SO261-34	23° 49.836' S	071° 22.534' W	7894.0	7500	-
3/12/18	Station 4	SO261-39	23° 21.965' S	071° 20.654' W	8061.3	50, 500, 2500, 5000	-
3/13/18	Station 4	SO261-40	23° 21.716' S	071° 20.614' W	8063.7	7700-7850	Yes
3/13/18	Station 4	SO261-47	23° 23.017' S	071° 20.699' W	8066.5	7000-?	Yes
3/16/18	Station 3	SO261-57	23° 03.065' S	071° 18.159' W	7992.1	5000	-
3/19/18	Station 7	SO261-74	22° 57.269' S	071° 37.243' W	5534.0	5400	Yes
3/21/18	Station 8	SO261-80	22° 56.803' S	072° 08.759' W	4118.1	2000, 4000	-
3/22/18	Station 2	SO261-82	21° 47.129' S	071° 12.482' W	7853.2	500, 2500, 5000, 6000	Yes
3/23/18	Station 2	SO261-91	21° 47.329' S	071° 12.337' W	7888.1	7800	-
3/24/18	Station 2	SO261-95	21° 47.880' S	071° 12.276' W	7879.9	6000	Yes
3/25/18	Station 10	SO261-100	20° 19.279' S	071° 17.463' W	7742.0	6000	Yes
3/27/18	Station 10	SO261-107	20° 19.574' S	071° 17.551' W	7743.3	7700	-
3/28/18	Station 9	SO261-110	20° 20.008' S	070° 59.395' W	4021.2	Niskin 3997, PRS 3950	Yes

Figure 1, below. Cell counts of four samples collected from the Atacama Trench that were fixed under *in situ* pressure without decompression (PRS) or after decompression collected within Niskin bottles (Niskin). Controls run using surficial seawater collected from Scripps pier show that fixation within the PRS at atmospheric pressure does not influence cell counts.

